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EFFECTS OF THE HERBICIDE IMAZAPYR ON BENTHIC MACROINVERTEBRATES IN A LOGGED POND CYPRESS DOME

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Abstract—Increased herbicide use in silviculture over the last several decades has led to concern over potential water contamination, which may affect biotic health. In the southeastern United States, pine flatwoods are important for timber production and are often interspersed with cypress wetlands. Cypress domes are isolated, shallow basins that collect surficial waters from adjacent forested areas and therefore might be expected to contain pesticide from storm runoff. This study utilizes in situ microcosm experiments to assess the effects of a concentration gradient of the herbicide imazapyr (0.184, 1.84, and 18.4 mg/L, equivalent to 1, 10, and 100 times the expected environmental concentration from a normal application rate) on the macroinvertebrate community of a logged pond cypress dome using changes in macroinvertebrate composition, chironomid biomass, and chironomid head-capsule deformities. The control core was not significantly different from the surrounding cypress dome for any parameter, suggesting that enclosure effects were likely of minimal importance in the final experimental results. The lack of statistical difference ($p < 0.05$) in macroinvertebrate community composition, chironomid deformity rate, and chironomid biomass between treatments suggests that imazapyr did not affect the macroinvertebrate community at the concentrations tested. Chironomid deformity rate ranged from 0.97% for imazapyr control to 4.96% for the 100× treatment, with chironomid biomass being 1.79 and 1.87 mg/L, respectively.

Keywords—Imazapyr Herbicide Macroinvertebrates Chironomid deformity Wetland

INTRODUCTION

Commercial forests cover vast areas in the United States and in the southeast are often composed of pine flatwoods. Most of the extensive deep-sand forested flatwoods of Florida (USA) are interspersed with cypress wetlands that serve as groundwater recharge areas. Herbicides are commonly used in these managed forests to reduce competition from noncommercial species, raising concern over potential water contamination. Cypress domes are basins that collect surficial waters from adjacent forested areas and therefore might contain pesticide from storm runoff. For this reason, these systems may show observable impacts.

Because of concern over potential herbicide contamination of surficial water, many herbicide fate studies have been conducted in southern pine forests [1–3]. Michael and Neary [4] and Neary et al. [5] reviewed literature on herbicide fate, dissipation, and environmental effects and found that few studies had examined herbicide toxicity on aquatic biota. Most toxicity studies have been laboratory bioassays that expose test organisms to high and static concentrations of compounds of interest for extended periods of time. Extrapolations of such toxicity data to field conditions are difficult because in situ exposures are often at highly variable and generally declining concentrations for most aquatic exposures.

Imazapyr [2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid], marketed as Arsenal Applicators Concentrate®, and Chopper® (BASF, Research Triangle Park, NC, USA) is a broad-spectrum herbicide used to control annual and perennial grass and broad-leaved weeds, vines, and deciduous trees in pine plantations and noncropland applications [6]. It is also used internationally to control brush in plantation crops such as sugar cane, rubber, and palm oil [7]. Imazapyr is an imidazolinone herbicide that interrupts production of branched-chain amino acids by inhibiting acetohydroxyacid synthase [8]. It is strongly adsorbed by soils and is usually found in the top few centimeters of soil [9], where it can remain active for two months to two years. Residual activity, when used at very high concentrations required for industrial weed control in Latin America, varies from two to three months in high rainfall tropical environments, 3 to 6 months in the subtropics, 6 to 12 months in humid temperate regions, and over 12 months in dry temperate areas [7].

Imazapyr is not currently designated for aquatic use by the U.S. Environmental Protection Agency. However, it can be transported into aquatic systems by drift and surface runoff. Herbicides may be lost from treated sites by a number of routes, including volatilization, hydrolysis, photolysis, and aerobic and anaerobic biodegradation. Given its high water solubility and low vapor pressure, imazapyr volatilization from water is not expected and degradation due to hydrolysis in the imidazolinone family is extremely slow. Mangels [10] found no detectable degradation of imazapyr due to hydrolysis up to 30 d in aqueous solution ranging from pH 5 to 7. Imazapyr is rapidly degraded by photolysis in water. Half lives are 1.9 to 2.3 d in distilled water, 2.7 d at pH 5, and 1.3 d at pH 9 [10]. Microbial degradation appears to be negligible. Mangels [10] found neither detectable anaerobic degradation of imazapyr for over one year nor aerobic degradation in 28 d for a sediment/water experiment.

Macroinvertebrates are commonly used as indicators of water quality because of their sensitivity to perturbations and

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position in aquatic and avian food webs [11]. Chironomids are typically the dominant macroinvertebrates in wetlands [12] and display a wide range of tolerances to environmental conditions. Deformities of chironomid head capsules have been linked to pesticides [13–16]. Such deformities could represent a chronic toxicity response and seriously impair the ability of the chironomid to feed. Biomass estimates of chironomids and other macroinvertebrates have also been used in pesticide studies in streams to address critical ecological questions [16,17]. Although previous studies examined effects of mixtures of toxins on chironomid deformities, relatively few have examined effects of toxins on chironomid biomass and almost none have used both as indicators of disturbance.

Few herbicide toxicity studies using macroinvertebrates have been conducted in freshwater wetlands [18], and examination of impacts on macroinvertebrates from imazapyr or other herbicides in the imidazolinone family is lacking. Microcosms have been used to evaluate the fate and effect of pesticides and increased nutrients at the individual, population, community, and ecosystem level. Laboratory microcosms are able to control environmental variables yet often poorly represent the targeted ecosystems. Limitations of these laboratory bioassays have been widely disputed [19,20]. In situ microcosms enable experimentation within natural systems, allowing testing of acute and chronic effects of toxins on individuals, communities of organisms, and ecosystem functions. This study utilizes in situ microcosm experiments to assess the effects of imazapyr on a macroinvertebrate community in a logged pond cypress dome using changes in macroinvertebrate composition, chironomid biomass, and deformities of chironomid head capsules.

METHODS

Study site

The study site was in a managed pine plantation approximately 40 km north of Gainesville (FL, USA) and consisted of a 30-ha block of pine flatwoods interspersed with pond cypress domes. The entire block was logged and replanted in slash pine (*Pinus elliottii* Engelm.) in 1994 [21]. The current microcosm study was conducted in a seasonally inundated, logged pond cypress dome with *Carex* spp. as the dominant emergent macrophyte. The typical pond cypress domes in the study site are inundated approximately 60% of the year, with mean pH of 4.1 and dissolved oxygen (DO) concentrations ranging from approximately 0.1 to 12 mg/L seasonally.

Microcosm design and field methods

In situ microcosms were used to test the toxicity of various concentrations of imazapyr. Each microcosm was a schedule-40 polyvinyl chloride water pipe (diameter ~7.62 cm; height 45.7 cm; area 45.6 cm^2) driven approximately 12 cm into the substrate and leaving a mean water column depth of 32.1 cm. Microcosms were installed and dosed with imazapyr on March 3, 1998, and left undisturbed for two weeks. Imazapyr treatments were assigned to a randomized complete-block experimental design, with all blocks having similar vegetation and water depth, which allowed a more strenuous statistical analysis. Forty-eight microcosms were divided into three blocks of 16 microcosms, and each block consisted of four replicates of three imazapyr treatments and a control microcosm without imazapyr (imazapyr control [IC]) each. Location and type of individual microcosms were assigned randomly. Treatments consisted of a gradient of three increasing concentrations of imazapyr. The final component of the block design included 12 randomly sampled cypress dome cores (CD), divided equally among the three blocks that were sampled at the end of the experiment. The CD cores were equivalent to microcosms. The CD treatment allowed us to test for microcosm influences on the measured parameters.

Imazapyr in the commercial form known as Arsenal Applicator's Concentrate (479 g acid equivalent/L) was obtained from a commercial supplier. Imazapyr was stirred into the water column of three microcosms in each replicate set to yield treatments of 1×, 10×, and 100× the expected environmental concentration, the concentration that should occur if the cypress dome (assuming the water depth in the microcosms at the beginning of the study) had been sprayed directly with the operational treatment rate for the surrounding forest site (0.56 kg acid equivalent/ha). Treatment concentrations were 0.184, 1.84, and 18.4 mg/L, respectively. Water samples were taken at both the time of application and the end of the experiment (14 d) to verify the initial imazapyr concentration and to calculate half-life values. At the end of the experiment, dissolved oxygen and temperature were measured using a dissolved-oxygen meter (YSI Incorporated, Yellow Springs, OH, USA) at the water surface and immediately above the water/substrate interface both inside and outside of four IC and four 100×-treated microcosms to assess microcosm and treatment effects. Continuous rain data were collected using a tipping bucket rain gage to determine possible herbicide dilution.

Following the two-week exposure period, microcosm (imazapyr treatment, IC, and CD) cores were collected. The cores (~5-cm diameter by 10-cm depth) were extracted by digging around the base of the polyvinyl chloride pipe, sliding the hand under the bottom of the core, and removing it to a bucket. The core of sediment and accompanying water column thus extracted were sieved through a 0.6-mm mesh. Macroinvertebrates and debris remaining on the mesh were then preserved in 80% ethanol and stained with approximately 10 mg/L of rose bengal for analysis. Additional rose bengal was added as needed to samples containing high concentrations of organic matter.

Laboratory methods

Imazapyr was determined in water by high-performance liquid chromatography using the method of Wells and Michael [22]. The mobile phase was acetic acid:water:acetonitrile (4:90:10 v/v) pumped at a flow rate of 1.5 ml/min. Column eluate was monitored at 240 nm. The method detection limit for the high performance liquid chromatography system was 1 μg/L, and the limits of quantitation varied according to injection volume: 5 μg/L for 200-μl, 10 μg/L for 100-μl, and 100 μg/L for 10-μl injections. Quality control samples consisted of blank and imazapyr-spiked pairs of water samples collected prior to treatment. Quality control samples were interspersed among field samples for analysis, and analytical standards were included as every fourth sample during routine analysis. Imazapyr half-life (the time to dissipation of 50% of the parent material) was calculated from water residue data. The value of half the limit of quantitation was substituted for nondetectable values, and the log of imazapyr concentration versus time was determined by simple linear regression. Half-life ($t_{1/2}$) was calculated from the slope of the regression line from the time of application [23].

Macroinvertebrates were hand picked from each sample and

Table 1. Mean (± standard error [SE]) water column temperature (°C) and dissolved oxygen concentration ([DOC] mg/L) of imazapyr control (IC) and 100× microcosms. Measurements were taken at the water surface and water–sediment interface (bottom) within the microcosms and in the surrounding water. $n = 4$ in each instance

Parameters	Inside top	Outside top	Inside bottom	Outside bottom
Temperature (IC)	20.5 (0.30)	19.1 (0.09)	13.9 (0.38)	14.4 (0.24)
Temperature (100×)	19.9 (0.26)	19.0 (0.09)	13.5 (0.29)	14.5 (0.18)
DOC (IC)	6.5 (0.55)	5.1 (0.18)	2.2 (0.31)	0.8 (0.24)
DOC (100×)	6.5 (0.38)	4.1 (0.40)	2.5 (0.39)	0.6 (0.10)

prepared for identification. Organisms other than chironomids were identified to family or lowest practical taxonomic level [24]. Total body lengths of chironomids were measured for estimating dry biomass, using a stereoscopic microscope with an ocular micrometer. Chironomids were then cleared with heated 5% potassium hydroxide solution, mounted using mounting medium (CMC-10; Masters Chemical, Elk Grove, IL, USA), and identified to genus using taxonomic keys [24,25]. Specimens of the chironomid genera *Chironomus*, *Kiefferulus*, *Polypedilum*, and *Procladius* were examined for mentum and ligula deformities. Definition of a deformity varies in the literature [26,27]. In this study, the mentum and ligula were considered deformed if they varied from the normal symmetrical configuration and were not damaged as a result of mounting [see also 13,28]. Natural breaks and wearing were not considered deformities due to a previous study by Madden et al. [15], which found an inverse relationship between natural breaks and wearing and toxicity while deformities increased with increased toxicity.

Biomass was determined from the total length-dry mass regression for the Chironomidae family by Benke et al. [29]. The power function $M = aL^b$ was used to predict dry mass, where M is the organism mass (mg), L is total length (mm), and a and b are constants (0.0018 and 2.617, respectively). The regression was calculated using mean a and b values from 17 previously calculated regressions of individual taxa within the family.

Statistical analysis

Two IC cores in one block were contaminated during imazapyr application, as verified by chemical analysis, and therefore were not used for analysis. Also, chironomid lengths from one CD core were not measured. All analyses were done using the SAS® Version 6.1 statistical package (SAS Institute, Cary, NC, USA). The significance level adopted for all comparisons was $p < 0.05$. Dissolved oxygen and temperature measurements were analyzed by both treatment and position (inside or outside of the microcosm) using two-way analysis of variance (ANOVA).

Taxa richness (total number of taxa) and abundance (total individuals) were calculated using only aquatic and semi-aquatic taxa. Specific taxa used in the mixed-model ANOVA were those macroinvertebrate orders displaying greater than 5% of total abundance and chironomid genera with greater than 5% of total chironomid abundance. Chironomid biomass was also analyzed using the mixed-model ANOVA (MIXED procedure from SAS Ver 6.1). This included the restricted maximum likelihood, an estimation procedure to test the significance of block variability, treatment × block interaction, and variation among microcosms nested within each block and treatment. Overall treatment effects (1×, 10×, 100×, IC, CD) were also tested within the mixed-model ANOVA. In addition, imazapyr treatment effects (1×, 10×, 100×, IC) were also analyzed with the mixed-model ANOVA, and only p values are shown. Fisher's least significant difference procedure was used to compare all possible treatment pairs.

Total chironomid deformities by treatment were analyzed using Fisher's exact test. A chi-square test was used to analyze combined treatments (1×, 10×, and 100×) versus IC. Since multiple genera were examined for deformities, it was necessary to account for any variability in deformities among genera. To do this, Fisher's exact test was used to analyze deformities by genus, ignoring treatment.

RESULTS

Imazapyr half-life

During the study, rain fell on three separate days after treatment (DAT). Assuming that rain diluted imazapyr within each microcosm according to the depth of the water column at the time of precipitation and the water surface intercept area, it was possible to calculate the extent of dilution. Mean imazapyr concentrations for treatments (1×, 10×, and 100×) were 0.19, 2.1, and 19.8 mg/L at time of application and declined to 0.001, 0.1, and 1.5 mg/L by the end of the experiment. The 35.1 mm of total precipitation (0.6 mm 2 DAT, 2.2 mm 4 DAT, and 32.3 mm 5 DAT) diluted the original treatments by approximately 11%. Diel flux in water-column depth potentially resulted in some dilution. Precipitation and diel stage flux are routine factors for determining field dissipation rates and, because they reduce the initial treatment concentration, result in a half-life that more accurately reflects conditions to which aquatic organisms are exposed. Variability caused by these two processes was reflected in the error terms for the regression analysis.

Half-life was calculated for the 1× (3.2 d), 10× (3.2 d), and 100× (3.4 d) treatments with regression r^2 values of 0.83, 0.94, and 0.89, respectively. Imazapyr is stable to hydrolysis, but it is very susceptible to photolysis and has a photolytic half-life of 2.7 d at pH 5 [10].

Shading of the microcosm water column by the polyvinyl chloride pipe walls was probably responsible for the somewhat longer half-life (3.2–3.4 d) observed for imazapyr than reported by Mangels [10] (2.7 d). Shading by microcosm walls reduced the light available for photolysis; thus, exposure of benthic macroinvertebrates in this study was somewhat longer than expected in this cypress dome.

Physical variables

Water temperature was statistically similar for the top and bottom of the IC and the 100× treatment microcosms (Table 1). Temperatures at the surface of the microcosms, however, were approximately a degree higher than the surrounding water and almost a degree cooler than surrounding water at the water–substrate interface. Although these differences were sta-

Table 2. Dominant taxa in the study. Taxa are listed in order of dominance. Specific chironomids were chosen based on their abundance (>5% of chironomid density)

Taxon	Percent of total abundance		
	Orders	Diptera	Chironomidae
Isopoda (*Caecidotea* sp.)	55.8		
Diptera	25.7		
Chironomidae		22.6	
Polypedilum			13.3
Chironomus			3.9
Ablabesmyia			1.4
Procladius			1.3
Other chironomids			2.7
Other diptera		3.1	
Amphipoda (*Crangonyx* sp.)	13.0		
Other taxa	5.5		
Total	100.0		

tistically significant ($p = 0.001$, f value = 28.24, and $p = 0.008$, f value = 9.44, respectively), they were probably biologically unimportant and most likely can be attributed to reduced water column mixing and reduced solar energy within the microcosms.

Dissolved oxygen was statistically similar for the top and bottom of the IC and 100× treatment microcosms (Table 1). However, dissolved oxygen was statistically different and greater by 1.3 to 2.3 mg/L inside the microcosms relative to the cypress dome at the surface ($p = 0.002$, f value = 24.18) and water–substrate interface ($p < 0.001$, f value = 45.95) (Table 1). The water–substrate interface was essentially anoxic (0.6 and 0.8 mg/L) outside the microcosm but hypoxic in the microcosm (2.15 and 2.45 mg/L).

Macroinvertebrate community composition and distribution

A total of 2,904 individuals representing 44 taxa were collected. The dominant orders were Isopoda (*Caecidotea* sp.), Diptera, and Amphipoda (*Crangonyx* sp.) in descending order, representing 94% of the total invertebrate abundance (Table 2). Chironomids were the principal dipterans and accounted for 22% of total abundance, with *Polypedilum*, *Chironomus*, *Ablabesmyia*, and *Procladius* being dominant.

Interblock and treatment-block interaction variability for taxon richness and total abundance for all analyzed taxa was very small compared with that among microcosms (Table 3). No significant differences were noted among blocks or treatment-block interactions. The large differences in the taxonomic richness and abundance of invertebrates among microcosms was expected due to both the natural heterogeneous distribution of benthic macroinvertebrates in swamps and the fact that the cypress dome had been logged, which caused greater heterogeneity of soil structure by mechanical mixing [30].

Table 4. Tests for treatment effects (fixed effects) examine overall treatments and imazapyr treatments. The p values for imazapyr treatments are from tests for treatment effects integrating only imazapyr (1×, 10×, 100×) treatments and imazapyr control (IC) and do not include cypress dome samples (CD). Total taxon richness (total number of taxa) and total abundance (total individuals) were calculated using only aquatic and semiaquatic taxa. The numerator $df = 4$ and the denominator $df = 8$ for overall treatment tests (1×, 10×, 100×, IC, CD) and numerator $df = 3$ and the denominator $df = 6$ for imazapyr treatment test (1×, 10×, 100×, IC)

Variable	Taxon	f Value	p Value	p Value, imazapyr treatment
Abundance	Total	1.04	0.45	0.64
	Caecidotea	1.86	0.21	0.89
	Crangonyx	0.72	0.60	0.58
	Dipteran	1.78	0.23	0.39
	Chironomid	1.33	0.34	0.44
	Polypedilum	1.46	0.30	0.84
	Chironomus	1.15	0.40	0.26
	Ablabesmyia	1.33	0.36	0.32
	Procladius	2.73	0.13	0.14
Taxa richness	Total	0.95	0.48	0.39
	Dipteran	0.59	0.68	0.63
	Chironomid	0.82	0.55	0.48

The mixed-model ANOVA tested for overall treatment effects (1×, 10×, 100×, IC, CD) and imazapyr treatment effects (1×, 10×, 100×, IC) (Table 4). No significant differences were found between treatments with respect to taxa richness and total abundance with or without the CD. All p values for a given parameter were similar with or without CD except for *Caecidotea*, with values of 0.21 and 0.89, respectively. The low abundance of *Caecidotea* in CD explains this difference. *Procladius* had the lowest p values with or without CD (0.13 and 0.14, respectively).

Total abundance of macroinvertebrates ranged from 42.6 individuals in CD to 64.4 in IC (Table 5). The abundances of dipteran, chironomid, *Caecidotea*, and *Chironomus* were highest in CD and IC treatments. Fisher's least significant difference analysis showed no significant difference in abundance

Table 3. Estimation of variation and standard error (SE) for block, treatment-block interaction, and microcosm (which are nested within each block and treatment) using restricted maximum likelihood procedure. Taxa are presented in order of dominance. Total taxon richness (total number of taxa) and total abundance (total individuals) were calculated using only aquatic and semiaquatic taxa

Variable	Taxon	Block		Treatment-block interaction		Microcosm	
		Variance (SE)	p Value	Variance (SE)	p Value	Variance (SE)	p Value
Abundance	Total	8.8 (32.2)	0.78	0 (0)	—	670.8 (133.0)	<0.0001
	Caecidotea	8.16 (28.5)	0.39	0 (0)	—	369.2 (73.2)	<0.0001
	Crangonyx	10.6 (12.3)	0.19	2.15 (4.17)	0.30	21.7 (4.83)	<0.0001
	Dipteran	0.0 (0)	—	0.0 (0)	—	35.9 (7.00)	<0.0001
	Chironomid	0.0 (0)	—	1.65 (5.02)	0.37	34.82 (7.47)	<0.0001
	Polypedilum	0.0 (0)	—	0 (0)	—	19.9 (3.94)	<0.0001
	Chironomus	0.2 (0.33)	0.32	0 (0)	—	2.10 (0.49)	<0.0001
	Ablabesmyia	0 (0)	—	0.26 (0.55)	0.32	0.95 (0.40)	<0.0001
	Procladius	0.05 (0.17)	0.37	0.11 (0.18)	0.28	0.43 (0.16)	<0.0001
Taxa richness	Total	0.02 (0.37)	0.47	0.78 (0.81)	0.16	3.10 (0.67)	<0.0001
	Dipteran	0.17 (0.38)	0.32	0.44 (0.50)	0.19	2.08 (0.45)	<0.0001

Table 5. Estimates for core means and standard error (±SE) in each treatment (1×, 10×, 100×), imazapyr control (IC), and cypress dome (CD) if every block had had an equal number of microcosms and multiple comparison procedure using Fisher's least significant difference. Significantly different ($p < 0.05$) treatment means between two treatments are shown in the last column with their p values. Total taxon richness (total number of taxa) and total abundance (total individuals) were calculated using only aquatic and semiaquatic taxa

		Treatments					Significant difference (p value)
	Taxon	CD	IC	1×	10×	100×	
Abundance	Total	42.6 (7.7)	64.4 (8.4)	49.2 (7.7)	52.8 (7.7)	54.6 (7.7)	
	Caecidotea	16.7 (5.8)	36.1 (6.3)	30.4 (5.8)	34.5 (5.8)	30.8 (5.8)	CD-IC (0.046)
	Crangonyx	8.3 (2.5)	8.8 (2.6)	5.8 (2.5)	5.6 (2.5)	7.1 (2.5)	
	Dipteran	16.1 (1.7)	15.0 (1.9)	11.5 (1.7)	10.5 (1.7)	13.7 (1.7)	
	Chironomid	14.3 (1.9)	13.2 (2.0)	10.7 (1.9)	8.8 (1.9)	12.0 (1.9)	
	Polypedilum	9.8 (1.3)	6.6 (1.4)	6.0 (1.3)	6.0 (1.3)	7.5 (1.3)	
	Chironomus	2.5 (0.6)	2.8 (0.5)	2.4 (0.5)	1.9 (0.06)	3.4 (0.05)	
	Ablabesmyia	1.5 (0.5)	2.5 (0.5)	2.5 (0.8)	1.0 (0.6)	1.4 (0.5)	
	Procladius	1.1 (0.5)	2.7 (0.4)	1.6 (0.3)	1.1 (0.3)	1.2 (0.4)	CD-IC (0.042), IC-10× (0.024), IC-100× (0.046)
Taxa richness	Total	8.5 (0.7)	9.5 (0.8)	7.7 (0.7)	8.1 (0.7)	8.9 (0.7)	
	Dipteran	4.9 (0.6)	5.3 (0.6)	4.3 (0.6)	4.3 (0.6)	4.7 (0.6)	
	Chironomid	3.8 (0.40)	4.1 (0.5)	3.7 (0.4)	3.1 (0.4)	3.4 (0.4)	

relative to imazapyr concentrations for any comparison except for *Caecidotea* and *Procladius*. *Caecidotea* control treatments CD and IC were significantly different ($p = 0.05$) from each other but not from the imazapyr treatments. For *Procladius* abundance, the IC and 10× treatments and the IC and 100× treatments were significantly different ($p = 0.02$ and $p = 0.05$), but the small numbers of individuals present in this study make interpretation of these results uncertain. The IC treatments were greater for both *Caecidotea* and *Procladius* abundance than for other treatments.

Total taxa richness means ranged from 7.7 in the 1× imazapyr treatment to 9.5 in the IC, with CD having a mean of 8.5. Differences in taxonomic richness were not statistically significant.

Chironomid deformities

We examined 573 chironomids for deformities (Table 6). While the proportion of total chironomid deformities appears slightly greater in the imazapyr-treated microcosms (1×, 10×, 100×) than in IC or CD, the differences were not significant. Neither intertreatment nor combined imazapyr treatment-imazapyr control ($df = 1$, coefficient of variation = 2.54) were significantly different.

Procladius had the highest natural proportion of deformity among the chironomids (independent of treatment) examined (7.9%; Table 7), and those were evenly distributed across the imazapyr treatments IC, 1×, and 10×. No deformities were observed among the 23 *Kiefferulus* specimens examined. No significant intergenus differences in deformity rates were observed.

Chironomid biomass

Dry-weight biomass (mg/L) estimates for total chironomid genera and their standard errors were CD 1.79 (0.54), IC 1.86 (0.51), 1× 1.42 (0.51), 10× 2.47 (0.51), and 100× 1.87 (0.51). Significant biomass differences were not found in either the mixed-model ANOVA testing for treatment effects ($p = 0.71$) or the pairwise Fisher's least significant difference procedure examining microcosm effects.

DISCUSSION

Limitations of single-species laboratory bioassays have been widely argued [19,20]. In situ microcosms enable experimentation at the individual, community, and ecosystem levels, which show acute and chronic effects at multiple trophic levels. Toxicity studies using both laboratory microcosms and natural systems often find greater deformity rates in laboratory controls than in natural controls. However, in situ microcosms can alter their environment by reducing interactions with the outside environment, water-column mixing, and light attenuation and increasing edge effects.

The benthic macroinvertebrate community in this cypress dome was similar to that of other wetlands studied at this location. Leslie et al. [21] identified 85 taxa from nine different cypress domes at this site over a two-year period, while we identified 44 different taxa from one sampling event. Chironomids typically are the principal macroinvertebrates in wetlands [12], and Leslie et al. [21] also found two chironomids, *Polypedilum* and *Chironomus*, as the dominant taxa at this site. The high density of *Caecidotea* in cypress domes is typ-

Table 6. Chironomid deformity listed by treatment (1×, 10×, 100×), imazapyr control (IC), and cypress dome (CD). No significant between treatment differences were found using Fisher's exact test ($p = 0.26$)

	Treatment					
	CD	IC	1×	10×	100×	Total
Number deformed	2	1	5	3	6	17
Number normal	141	102	105	93	115	556
Percent deformed	1.4	0.97	4.55	3.1	4.96	2.97
Total number examined	143	103	110	96	121	573

Table 7. Deformities listed by Chironomid genera and ignoring treatment. No significant intergenus differences were found using Fisher's exact test ($p = 0.22$)

	Chironomus	*Kiefferulus*	*Polypedilum*	*Procladius*	Total
Number deformed	4	0	10	3	17
Number normal	111	23	387	35	556
Percent deformed	3.45	0	2.52	7.89	2.97
Total number examined	115	23	397	38	573

ical and likely reflects successional changes following logging [21].

Microcosms in this study appear to be valid mimics of the cypress dome's physical properties, macroinvertebrate composition, and chironomid deformity and biomass. The statistically significant temperature and DO differences between the treated microcosms and the surrounding water in this study were small and probably had little effect on imazapyr half-life and macroinvertebrates. The greater DO concentrations within the microcosms could reduce stress on macroinvertebrates.

Microcosms had little effect on macroinvertebrate community composition, chironomid deformity rate, and chironomid biomass. The IC and CD treatments were similar for all individual taxon abundance estimates except *Caecidotea* and *Procladius*, where the IC treatment had greater abundance than all other treatments. The latter was probably due to the abundance of *Caecidotea* in that treatment. *Procladius* was the least abundant taxon sampled, which could have led to errors based on the small population size. Chironomid deformities and biomass were similar in IC and CD, suggesting little change in trophic interactions within the microcosm.

Aquatic invertebrate responses to imazapyr and the imidazolinone family have been limited to 48-h median lethal concentration (LC50) acute toxicity tests using *Daphnia magna*. The order of toxicity to *D. magna* in the imidazolinone family, from greatest to least, is imazapyr, imazamethabenzmethyl, imazaquin, and imazethapyr, with LC50 acute toxicity of >100 mg/L, 220 mg/L, 280 mg/L, and >1,000 mg/L, respectively [31]. Imazapyr and the imidazolinone family have low toxicity potentials similar to those of herbicides like hexazinone (LC50 = 152 mg/L), triclopyr technical (LC50 = 133 mg/L), and glyphosate technical (LC50 = 780 mg/L). Other herbicides with greater *D. magna* toxicity relative to the imidazolinone family include 2,4-D-amine (LC50 = 4 mg/L) and 2,4-D-ester (LC50 = 1.2 mg/L) [32].

There are no other published studies of impacts of imazapyr or other herbicides in the Imidazolinone family on the community structure of benthic macroinvertebrates. This study shows that imazapyr had little effect on the macroinvertebrate community, specifically total taxa richness and abundance. There was also no evidence for effect on the abundance of total dipterans and chironomids and that of individual macroinvertebrate genera *Caecidotea*, *Crangonyx*, *Polypedilum*, *Chironomus*, and *Ablabesmyia*.

The frequency of total chironomid deformities was not statistically different among treatments. The low number of deformities and the lack of any perceptible effect on abundance or richness in the 100× microcosms suggest that imazapyr probably is not toxic to macroinvertebrates found in these cypress domes. Other studies have found deformity rates of up to 5% in reference [28,33,34] systems. Madden et al. [14] found deformity rates of 19% for *Chironomus* and 8% for *Dicrotendipes* in control laboratory tanks. Pettigrove et al. [35] found greater deformity associated with *Polypedilum* and *Procladius* (34.2 and 16.8%, respectively, of all abnormalities) when examining rice bays treated with the insecticides malathion and chlorpyrifos and the herbicides molinate and bensulfuron. They also found 41.6% of total chironomid deformities in treated rice fields and only 19.7% in reference sites. However, Madden et al. [15] showed that dacthal induced head capsule deformities, but clear dose–response curves could not be detected.

Imazapyr also appears to have little effect on chironomid biomass via direct growth inhibition or secondary means (i.e., reduced eating due to mouthpart deformities). Similarly, Schneider et al. [17] found macroinvertebrate biomass and periphyton were not affected when examining the herbicide hexazinone in outdoor stream-channel experiments. However, insecticides like methoxychlor can reduce insect abundance by 75% in mountain streams and greatly reduce macroinvertebrate biomass [16].

Other herbicide studies examining effects on macroinvertebrates have ranged from no effect on populations to decreased and increased abundance as well as acute and chronic toxicity. Many herbicides used in forestry and agriculture have been tested for effects on biota in streams but rarely in wetlands. Linz et al. [36] examined the effects of glyphosate in prairie pothole wetlands. They found a significant increase in macroinvertebrate and chironomid abundance in treated wetlands and suggested that this was an indirect effect caused by increased food resources resulting from cattails (*Typha* spp.) being killed by the herbicide. Atrazine caused a significant reduction in gross primary productivity and aquatic invertebrate community structure in northern pothole prairie simulation microcosms [37]. Using stream microcosms, Solomon et al. [38] suggested that atrazine did not disrupt ecosystem structure and function below a concentration of 5 mg/L. However, Kiesecker [39] demonstrated exposure to trematode infection was required for development of limb deformities in *Rana sylvatica*, but concurrent exposure to pesticides (esfenvalerate, malathion, or atrazine) appeared to act synergistically, increasing the rate of occurrence. Pesticide exposure in the absence of trematode infection did not result in amphibian limb deformity. Hexazinone has been studied in forested watersheds in Georgia [40] and the Alabama piedmont (USA) [41], and neither study found a significant difference in macroinvertebrate species composition or diversity when exposed to 44 and 473 μg/L of hexazinone, respectively. Cuppen et al. [42] observed a significant increase in *Caecidotea* abundance in microcosms treated with 50 and 150 mg/L linuron. The current imazapyr study not only adds to the community response of macroinvertebrates to pesticides in wetlands, it also explores potential chronic responses to chemicals through use of chironomid head capsule deformity rates and chironomid biomass. This study suggests that imazapyr does not have acute or chronic effects on macroinvertebrate composition, chiron-

omid deformity rates, or biomass at concentrations up to 100 times the expected environmental concentration and has low toxicity compared with many other herbicides used in forestry and agriculture. It is unlikely to pose a risk of harm to aquatic invertebrates when used in forest vegetation management at prescribed rates and at the normal frequency of one to three times in a rotation of 20 to 80 years.

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